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Enhanced enzymatic digestibility of bamboo by a combined system of multiple steam explosion and alkaline treatments

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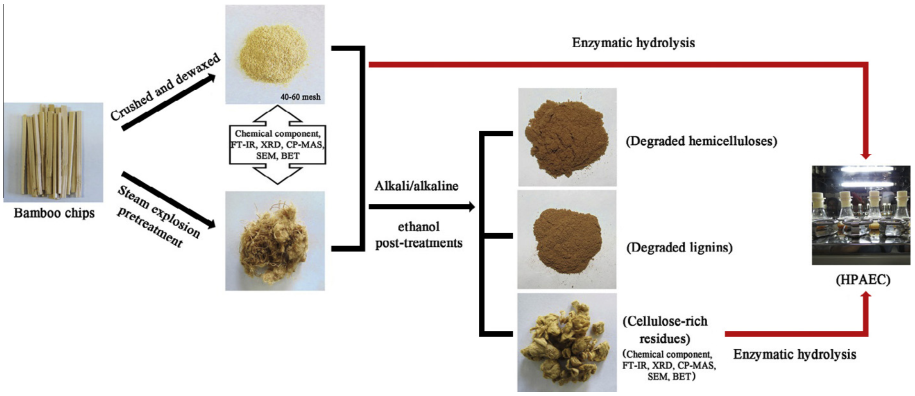
HIGHLIGHTS

- Combined system based on steam explosion and alkaline treatment was developed.
- The maximum enzymatic digestibility of the pretreated bamboo reached 73.8%.
- The removal of hemicelluloses and lignin facilitates bamboo enzymatic digestibility.

GRAPHICAL ABSTRACT


Bamboo chips
Crushed and dewaxed
40-60 mesh
Chemical component, FT-IR, XRD, CP-MAS, SEM, BET
Steam explosion Pretreatment
Enzymatic hydrolysis
Alkali/alkaline ethanol post-treatments
(Degraded hemicelluloses)
(Degraded lignins)
(Cellulose-rich residues)
(Chemical component, FT-IR, XRD, CP-MAS, SEM, BET)
Enzymatic hydrolysis
(HPAEC)

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ABSTRACT

A combined system based on multiple steam explosion pretreatments (direct pretreatment and presoaked in 1% KOH aqueous solution followed by pretreatment under different conditions) and mild alkaline post-treatment has been developed to obtain digestible substrates from bamboo stems for bioethanol production. After the pretreatments, the enzymatic digestibility of cellulose increased to 17.1–32.2%, as compared to that of the untreated bamboo stems (5.8%). Direct pretreatment followed by alkaline treatment increased the enzymatic digestibility of cellulose to a maximum value of 73.8%. Alkaline treatment removed most of lignin and hemicelluloses, and incurred a higher crystalline index of the cellulose-rich residue obtained after a synergistic treatment as compared to the only steam-exploded substrates. The combination of direct pretreatment and alkaline treatment is an environmentally friendly and economical feasible method for the production of glucose and high-purity lignin, which will be further converted into high value-added products based on biorefinery.

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1. Introduction

Lignocellulosic materials are the only sustainable and renewable resources that can provide alternative of crude oil, fossil fuels, etc [1–3]. The conversion of abundant lignocelluloses to biofuels presents a viable option for enhancing energy security and reducing greenhouse gas (mainly carbon dioxide) emissions. *Phyllostachys pubescens*, belonging to *Bambusoideae* of *Gramineae*, is a bamboo species with the highest economic value in China and mainly distributed in the south of the Yangtze River. Because of its easy propagation, rapid growth, and high productivity, *P. pubescens* is considered as one of the most potential renewable non-woody lignocelluloses for biofuels production in biorefinery industry [4].

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Unfortunately, although the biochemical conversion of lignocelluloses into biofuels has significant economical and technical potential, lignocellulosic materials naturally resist enzymatic attack due to many physicochemical factors, such as the existence of lignin and hemicelluloses, cellulose crystallinity, accessible surface, and the presence of covalent cross-linkages between lignin and hemicelluloses [5]. Thus, an efficient pretreatment system must be applied prior to the enzymatic hydrolysis to overcome recalcitrance of lignocelluloses and improve the accessibility of cellulases to the pretreated substrates [6].

For the past few years, several promising pretreatment technologies have been developed, such as steam explosion, hydrothermal, and organosolv pretreatments [7–9]. Among these technologies, steam explosion pretreatment (SEP) is the utmost frequently used approach [10]. In this case, chipped lignocelluloses are treated with high saturated vapor pressure and then the pressure is rapidly reduced, which makes the material more bouffant, resulting in an easier accessibility to enzymes through explosive decompression. SEP is typically initiated at a pressure of 0.69–4.83 MPa from several seconds to a few minutes before the material is exposed to atmospheric pressure. In addition, the process leads to hemicelluloses degradation and lignin transformation at a high temperature, and makes the cellulose more accessible to enzymatic attack, therefore increasing the potential of biomass hydrolysis [11]. Moreover, an important step which influences the effect of SEP is whether to presoak lignocelluloses before SEP. The main purpose of presoaking is to soften fiber, which makes the fiber more free from mechanical damage. In general, aqueous alkaline solution is considered to possess strong penetrating ability to make fiber swell [12,13]. It should be noted that the aqueous solutions of potassium, sodium, and lithium hydroxide are appropriate for extraction hemicelluloses from lignocellulosic materials, but the preferred alkali was potassium hydroxide since potassium acetate formed during the neutralization is more soluble in ethanol used for precipitation than other acetates [14]. Meanwhile, in order to make the alkaline liquor can completely infiltrate into the biomass, the pre-soaking was generally performed at a long period of time and a low temperature (at room temperature for 24 h). On the other hand, if the pro-soaking is performed at high temperatures, it normally needs appropriate acid-resistant equipment and high energy density. This undoubtedly increases the cost of the entire process.

Although SEP process is a promising technology for lignocelluloses, some disadvantages, such as only partial degradation and removal of hemicelluloses and lignin, as well as incomplete disruption of lignin-hemicelluloses matrix will impede the enzymatic digestibility of pretreated substrate. Therefore, a further post-treatment is required to remove lignin and degraded hemicellulosic products from the steam-exploded substrates so as to improve enzymatic digestibility of the pretreated substrates. It has been reported that alkaline treatment is a promising technology for the effective removal of hemicelluloses and lignin (delignification), which can dramatically enhance enzymatic digestibility of substrate [10,15,16]. Among these alkaline regents, NaOH exhibits the most significant effect on degrading lignin and improving subsequent fermentation yields [17].

Due to a high degree of lignification in the cell walls of bamboo, only a few studies for improving the efficiency of enzymatic hydrolysis of bamboo have been developed by single or combined treatment procedures [18–20]. As compared to other pretreatments, steam explosion pretreatment (SEP) has been successfully applied in some bio-ethanol plants in China. However, some improvements still need to be done to achieve an economic biorefinery aim, for example, to investigate the influence of this promising pretreatment on enzymatic hydrolysis efficiency of bamboo. In addition, exploration of potential utilization of hemicelluloses and/or lignin obtained from the combined process is of great importance to achieve an economically feasible biorefiery industry. Based on the synthetic consideration, a combined system consisting of SEP and mild alkaline post-treatment has been developed to explore bamboo stems for the improvement of its enzymatic digestibility in the present study. As a biorefinery process, hemicelluloses and/or lignin attracted close attentions for its potential value in developing bio-based materials and chemicals. Meanwhile, the structure features and potential utilization of the hemicelluloses and lignin obtained from alkaline post-treatment will be investigated further. In short, all the results will provide useful information in the utilization of bamboo stems for large-scale enzymatic hydrolysis in the prospective biorefinery industry.

2. Experimental

2.1. Material

Bamboo stems (*P. pubescen*) were obtained from Jiangxi province, China. They were first dried in an oven at 60 °C for 24 h and then ground in a mill to obtain a 40–60 mesh fraction. Then the particle was extracted with toluene–ethanol (2:1, v/v) in a Soxhlet apparatus for 6 h to remove extractives (approximate 10.92%) and then dried in an oven at 60 °C for 24 h to serve as control substrate (substrate S1).

2.2. Steam explosion pretreatment (SEP)

Bamboo stems were cut into chips with an average size of around 100 mm × 15 mm × 15 mm. The chips were divided into four batches (each 500 g). One batch was conducted by the direct SEP at 2.0 MPa for 5 min (substrate S2, without presoaking), while other three batches were first pre-soaked in 1% KOH aqueous solution for 24 h at room temperature (25 °C) followed by SEP at 1.8 MPa and 2.0 MPa for 5 min (substrates S3 and S4) and 2.0 MPa for 8 min (substrate S5), respectively.

2.3. Alkaline post-treatment

The substrates S1, S2, S3, S4, and S5 (10 g) were successively post-treated with 0.5% NaOH aqueous solution and 60% aqueous ethanol containing 1.5% NaOH at 80 °C for 3 h with a solid to liquor ratio of 1:25 (g/mL), and the corresponding solid residues were named as R1, R2, R3, R4, and R5, respectively. The details of the successive extractions were described in the previous literature [21]. Meanwhile, the yield, purity, dissociation mechanisms, structural features, and structural transformations of the obtained lignins were thoroughly investigated [21]. In this study, all the prepared S substrates (S1, S2, S3, S4, and S5) and R substrates (R1, R2, R3, R4, and R5) were applied to produce glucose by enzymatic hydrolysis in the present experiment.

2.4. Enzymatic hydrolysis

The experiments were carried out at 2% of substrate (w/v) in 10 mL of 50 mM sodium acetate buffer (pH 4.8) using a double-layer shaking incubators (ZWYR-2102C) (Shanghai, China) at 150 rpm for 72 h. The temperature was controlled at 50 °C. Cellulase (15 FPU/g substrate), which was supplied by Shanghai Youtell Biochemical Co., Ltd., was used for all the hydrolysis experiments. The hydrolyzates were analyzed by a high performance anion exchange chromatography (HPAEC) system (Dionex, ICS 3000, USA) on a CarboPac PA 100 analytical column as described elsewhere [22]. All the enzymatic hydrolysis experiments were performed in triplicate, and average values and corresponding derivations were given.

2.5. Analysis methods

The chemical compositions (%, w/w) of all the substrates were determined by National Renewable Energy Laboratory's (NREL) standard analytical procedure [23]. Analyses of the carbohydrates of all the substrates were conducted by a HPAEC system on a CarboPac PA 20 (Dionex, U.S.) analytical column according to the literature [24]. Fourier transform infrared (FT-IR) spectroscopic measurements were performed on a Nicolet iN10 spectrophotometer in the range from 1800 to 800 cm^{-1} at 4 cm^{-1} resolutions with 64 scans. X-ray diffraction (XRD) in reflection mode was recorded using an XRD-6000 apparatus (Shimadzu, Japan) with Nifiltered Cu Kα radiation (χ = 1.54 Å) generated at 40 kV and 30 mA. The scattering angle (2θ) was from 5 to 35° at a scanning speed of 2°/min. The crystallinity indexes (CrIs) of the substrates were determined from the ratio of the crystalline peak area to the total area of crystalline and amorphous peaks. Solid-state cross-polarization/magic angle spinning (CP/MAS) ^{13}C NMR spectra of the substrates were obtained at 100.6 MHz using a Bruker AV-III 400 M spectrometer (Germany). Scanning electron microscopy (SEM) images were performed with a Hitachi S-3400N II (Hitachi, Japan) instrument at 10 kV and 81 mA. All the substrates were coated with gold prior to examination. Brunauer–Emmett–Teller (BET) surface areas and Barrett–Joyner–Halenda (BJH) pore volumes of the substrates were recorded by analyzing of the nitrogen adsorption using a TriStar 3000 surface area analyzer (Micromeritics Ltd., Tokyo, Japan) after 8 h of degassing at 120 °C and 1 h of degassing at 150 °C.

3. Results and discussion

3.1. Mass changes of bamboo stems during the combined system

The combined system of multiple SEP and successive alkaline treatments was applied to enhance enzymatic digestibility of bamboo stems. SEP of chipped bamboo stems were performed in a sealing reactor. After the SEP, only solid residues were recovered. During the SEP, most of hemicelluloses were degraded into oligosaccharides and other small molecules, which were collected with the steam-exploded bamboo before sequential alkaline post-treatment. An increase in the alkaline concentrations can result in a more release of lignin embedded compactly in the lignocellulosic matrix [21]. Hence, a successive alkaline post-treatment (0.5% NaOH aqueous solution and 60% aqueous ethanol containing 1.5% NaOH) was applied to significantly remove lignin and the degraded hemicellulosic products from the steam-exploded substrates. In this study, the yields of residues obtained from the untreated and steam-exploded residues after alkaline treatment are shown in Table 1. As can be seen, the yield of residues ranged from 66.2% to 72.2%, in which only 66.2% residue was obtained from S2. This is mainly because more lignin was removed by alkaline treatment when bamboo stems were conducted at 2.0 MPa for 5 min without presoaking as compared with those obtained at other SEP conditions. For the presoaked bamboo, the yield of residue gradually decreased with the pretreatment severity increased, which was probably related to the removal of lignin (Table 1).

Table 1
Yields of cellulose-rich residues and lignin obtained from the untreated and steam-exploded residues after alkali/alkaline-ethanol treatment.

	R1	R2	R3	R4	R5
Yield (%)	72.2	66.2	71.7	69.3	68.1
Lignin (%)	8.7	17.6	9.3	13.4	15.1

3.2. Compositional analysis

To obtain a higher yield of glucose for bioethanol production by enzymatic hydrolysis of the cellulose-rich substrates, lignin in the pretreated substrates must be extensively removed prior to enzymatic hydrolysis. Therefore, a two-step alkaline procedure was developed to remove the lignins from the steam-exploded substrates for the subsequent bioethanol production. The composition of the extractive-free bamboo, which was determined by the NREL standard analytical procedure [23], was moisture 5.26% and lignin 27.53% (25.12% Klason lignin and 2.41% acid-soluble lignin). Cellulose (expressed as glucan, 39.75%) was the main constituent in the untreated substrate (S1). Hemicelluloses were composed of xylan, arabinan, and galactan, accounting for 24.73%. The chemical compositions of the steam-exploded substrates (S2, S3, S4, and S5) and alkali/alkaline ethanol post-treated substrates (R1, R2, R3, R4, and R5) were determined and shown in Table 2.

SEP is extensively applied to degrade hemicelluloses and lignin, disrupt the lignocellulosic structure, and to increase enzyme accessibility of the substrate. The goal of pretreatment is to remove hemicelluloses and/or lignin since they could inhibit enzymatic hydrolysis of biomass [25]. The main degraded components during the SEP process were hemicelluloses and lignin, which was manifested in Table 2. After SEP, the contents of hemicelluloses (8.53–18.00%) and Klason lignin (21.06–23.82%) of the pretreated substrates obviously decreased as compared to the untreated substrate, especially for hemicelluloses. Specially, S2 contained relatively higher cellulose (52.51%), but lower hemicelluloses (8.53%) and Klason lignin (21.08%) as compared to S3, S4, and S5, suggesting that direct SEP was more effective to degrade hemicelluloses and lignin than SEP with alkaline presoaking under the conditions given. As the pretreatment severity increased, the contents of hemicelluloses and Klason lignin gradually decreased, indicating that the degradation degree of hemicelluloses and lignin increased with the raise of pretreatment severity. After the further alkaline treatment, R substrates with abundant cellulose (51.79–70.08%) and small amounts of hemicelluloses (6.33–18.61%) as well as Klason lignin (6.76–15.81%) were obtained. Particularly, only 6.76% Klason lignin and 6.33% hemicelluloses were detected in R2. As compared with S substrates, a similar variation trend was observed in R substrates, i.e., the contents of lignin and hemicelluloses decreased steadily with the increased pretreatment severity. These results indicated that the SEP combined with alkaline treatment was an effective method to remove lignin and hemicelluloses, especially under direct SEP condition without presoaking.

3.3. Fourier transform infrared (FT-IR)

FT-IR spectroscopy was used to investigate the structural changes of the steam-exploded and post-treated bamboo stems (Fig. S1). An intensive band at 1732 cm^{-1} (stretching of C=O in hemicelluloses) was observed in the spectrum of the untreated substrate [26]. However, this band was inconspicuous in the spectra of the steam-exploded and alkaline post-treated substrates, suggesting the partial degradation of hemicelluloses during SEP and alkaline treatment. Generally, the hydrolysis of glycosidic linkages in hemicelluloses is catalyzed by acetic acid originated from acetyl groups in hemicelluloses at higher temperatures [27]. It was found that the spectra of R substrates showed more intense band at 1653 cm^{-1} than S substrates, which was ascribed to more oxidized lignin units bearing carbonyl groups at C_α in R substrates. Moreover, several strong bands could be clearly observed in the spectra of S substrates: the bands at 1602, 1506, and 1422 cm^{-1}, corresponding to aromatic skeletal vibrations, and the bands at 1460 and 824 cm^{-1} represent the C—H deformation combined with aromatic ring vibration and aromatic C—H in-plane deformation (typically for S

Table 2
Chemical compositions of the untreated (S1), steam-exploded (S2, S3, S4, and S5), and further alkali/alkaline ethanol post-treated (R1, R2, R3, R4, and R5) substrates.

Entry	Substrate composition[a] (w/w, %)					
	Arabinan	Galactan	Glucan	Xylan	KL	ASL
S1	1.42 ± 0.04	0.37 ± 0.02	39.75 ± 1.56	22.94 ± 0.87	25.12 ± 0.95	2.41 ± 0.10
S2	ND[b]	ND	52.51 ± 1.77	8.53 ± 0.28	21.08 ± 0.83	1.82 ± 0.07
S3	1.09 ± 0.03	0.19 ± 0.01	42.62 ± 1.63	16.72 ± 0.37	23.82 ± 0.91	2.18 ± 0.08
S4	0.93 ± 0.03	0.23 ± 0.02	44.76 ± 1.82	15.14 ± 0.33	22.1 ± 1.07	2.32 ± 0.09
S5	0.42 ± 0.02	0.21 ± 0.01	46.84 ± 1.34	14.73 ± 0.27	21.63 ± 0.95	2.22 ± 0.05
R1	1.29 ± 0.05	0.18 ± 0.01	51.79 ± 1.97	17.14 ± 0.53	15.81 ± 0.56	2.04 ± 0.06
R2	ND	ND	68.53 ± 2.11	6.33 ± 0.21	6.76 ± 0.31	0.89 ± 0.03
R3	1.14 ± 0.04	ND	54.42 ± 1.73	16.25 ± 0.45	11.63 ± 0.52	1.53 ± 0.07
R4	0.83 ± 0.02	ND	61.11 ± 1.82	15.43 ± 0.61	9.68 ± 0.47	1.34 ± 0.06
R5	ND	ND	70.08 ± 2.23	8.03 ± 0.37	7.82 ± 0.36	1.04 ± 0.04

[a] Cellulose was expressed as glucan and hemicelluloses was expressed as xylan, arabinan, and galactan. KL was Klason lignin (i.e. acid insoluble lignin) while ASL was acid soluble lignin.

[b] ND, not detected.

units), respectively [28]. However, the intensities of these bands sharply decreased in the spectra of R substrates, indicating the effective removal of lignin during alkaline post-treatment process. In addition, an intensive band at 894 cm^{-1} (β-glycosidic linkages between glucose unites in cellulose) was observed in the spectra of R substrates, suggesting the increment of cellulose content in the alkaline post-treated substrates as compared to the untreated and only steam-exploded substrates. In short, the data presented here suggested that the combined system was an effective method for removal of hemicelluloses and lignin from bamboo stems.

3.4. Crystallinity of substrate

Among the various structural characteristics affecting enzymatic digestibility of biomass, the crystallinity of cellulose is identified as one of the main substrate features that influence hydrolysis kinetics and yields [29]. In the present study, XRD and solid ^{13}C CP/MAS NMR spectroscopic techniques were applied to determine CrIs of all the substrates. Fig. S2 shows the XRD patterns of the untreated, steam-exploded, and alkaline post-treated substrates. The peaks of crystalline cellulose appeared at approximately 16.5° and 22.5°, and the region around 18.5° was due to the amorphous cellulose. The CrIs were calculated from XRD patterns and are shown in Fig. S2. After SEP, the CrIs of the pretreated substrates slightly increased to different extents, which was certificated by the increasing intensity of peak located at $2\theta = 22.5°$. The CrI of the untreated substrate was 41.57%, while the CrIs of the steam-exploded substrates increased to 42.94–45.60%, suggesting that some amorphous hemicelluloses were degraded during the SEP, as also revealed by the decreased intensity of band at 1734 cm^{-1} in the FT-IR spectra. The increased CrI indicated that CrI of the substrate was related to the severity of SEP condition. Further alkaline treatment resulted in the substrates with higher CrIs values, which was attributed to the significant removal of the non-crystalline hemicelluloses and lignin during the alkaline post-treatment, as evidenced by the aforementioned FT-IR spectra and compositional analyses.

Solid-state CP/MAS ^{13}C NMR technique, which can provide detailed insights into the chain conformation straightway without chemical and physical changes, is a powerful tool to investigate the structural changes of the substrates induced by the combined treatment. The CP/MAS ^{13}C NMR spectra of S and R substrates are shown in Fig. 1. All noticeable signals are distributed in the region of 60.0–110.0 ppm and originated from the carbons of carbohydrate polymer, mainly cellulose. The region between 86.0 and 92.0 ppm is derived from the crystalline forms and paracrystalline of cellulose (the ordered region), whereas amorphous regions of cellulose are distributed in the region of 80.0–86.0 ppm. An additional signal observed at 55.5 ppm (OCH$_3$) indicated that in the presence of lignin in the substrates [30]. As shown in Fig. 1, the untreated substrate had a relatively low CrI of 28.41%, while the CrIs of the steam-exploded substrates slightly increased to 34.60–39.06%. After further alkaline post-treatment, the CrIs of the post-treated substrates further increased to 35.97–43.86%. These results were consistent with CrI values from XRD data. In addition, it was found that the signal at 55.5 ppm was almost disappeared in R substrates, implying that residual lignin was almost removed after the integrated process.

3.5. Surface morphology and BET surface area

SEP could significantly alter the morphology of biomass, which also reflects the accessibility of the substrates [31]. To investigate the morphology changes caused by SEP and alkaline treatments, SEM images of the untreated, steam-exploded, and post-treated substrates were observed at magnifications of 1000 (Fig. 2). The untreated substrate displayed a rigid and rough surface morphology, which could impede the accessibility of cellulose to enzymes [32]. By contrast, the surfaces of the steam-exploded substrates were broken into separated fibers or fiber bundles and appeared slight cracks and small particle-sized debris. Notably, the surface morphology of the direct steam-exploded substrate became looser with wider separation of the fibers than the steam-exploded substrates with KOH presoaking. All these changes led to large amounts of reactive sites on the fiber surface, thereby increasing the accessibility of enzymes and enhancing the enzymatic hydrolysis efficiency. After alkaline post-treatment, the surfaces of R substrates became smoother as compared to the corresponding S substrates, suggesting that lignin and hemicelluloses were significantly removed by the alkaline treatment.

SEP and alkaline post-treatment also led to the changes of BET surface areas and BJH pore volumes of the substrates (Table 3). It is recognized that the increment of surface area and pore volume enhanced accessibility of enzymes to cellulose and therefore improved the enzymatic digestibility of cellulose [33]. In this study, the steam-exploded substrates showed relatively higher surface areas as compared to the untreated substrate. In addition, the surface areas of the steam-exploded substrates pretreated with KOH presoaking increased with the severity of pretreatment, however, which are lower than that of the direct steam-exploded substrate. This increased trend was well correlated with partial breakdown of the microstructure of the substrate particles. The existence of such a loose structure would be desirable to enzymatic hydrolysis of biomass because it would allow extensive access of cellulases to the surfaces of the substrates. Furthermore, the surface area and pore volume simultaneously reached the maximum

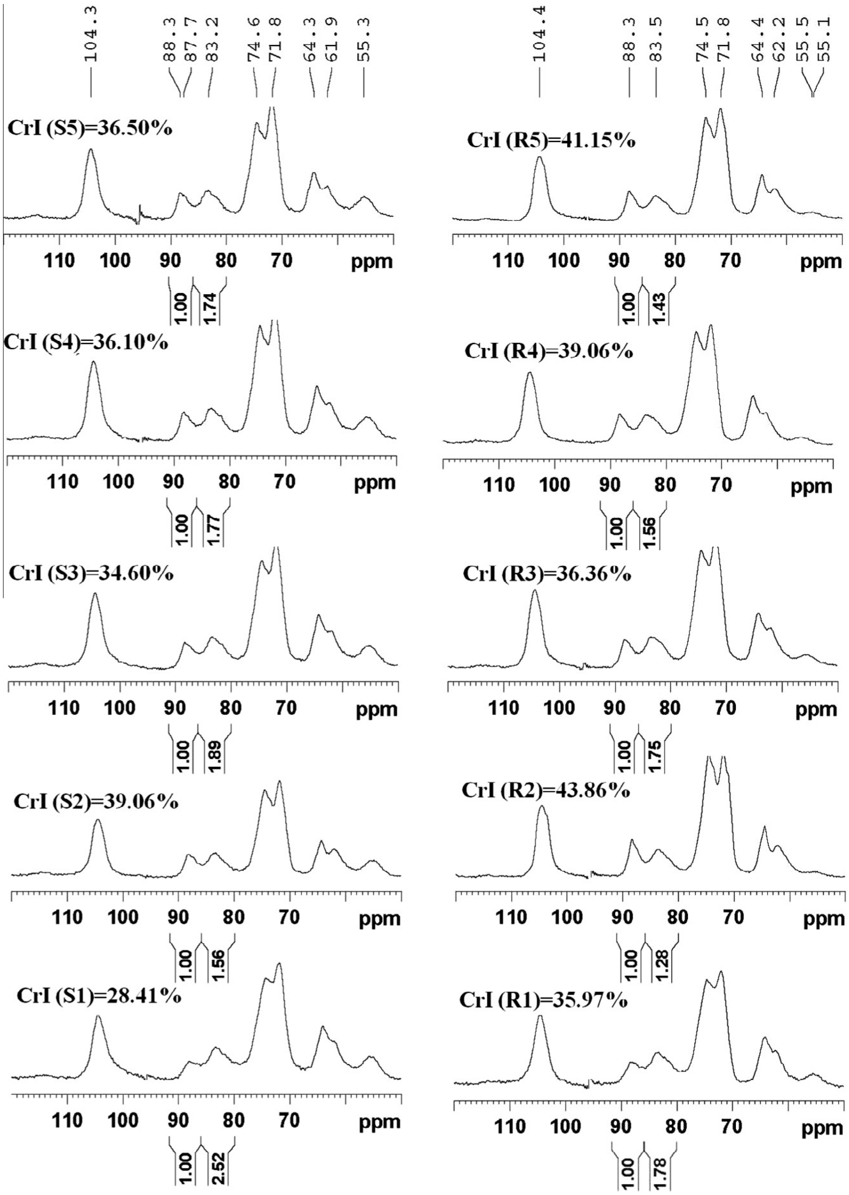

Fig. 1. CP/MAS ^{13}C NMR spectra of the untreated, steam-exploded, and further alkali/alkaline ethanol post-treated substrates.

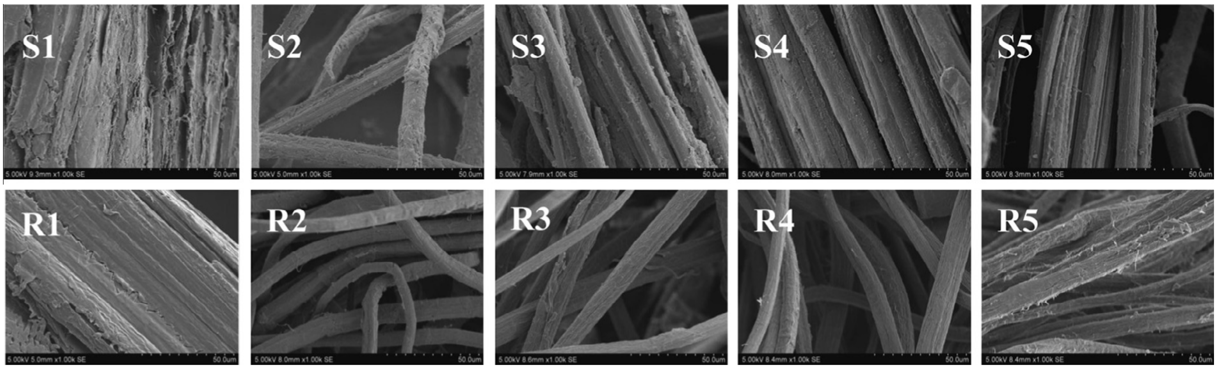

Fig. 2. SEM images of the untreated, steam-exploded, and further alkali/alkaline ethanol post-treated substrates (magnification is 1000).

values for the substrates pretreated under direct SEP condition, as revealed by the aforementioned analysis (Table 3). For the alkaline post-treated substrates, similar results were detected, that was, the surface area increased gradually with the increased pretreatment severity. As expected, the maximum surface area and pore volume was simultaneously reached in R2. The enhanced surface area and

Table 3
BET surface area (m^2/g) and BJH pore volume (cm^3/g) of substrates analyzed by BET method.

	Substrates									
	S1	S2	S3	S4	S5	R1	R2	R3	R4	R5
BET surface area	0.92	1.20	1.09	1.12	1.18	0.54	1.20	0.34	0.78	0.91
BJH pore volume	0.003	0.006	0.003	0.003	0.003	0.001	0.005	0.002	0.003	0.003

pore volume potentially resulted in the increased digestibility of the pretreated substrates.

3.6. Enzymatic hydrolysis

The amenability of the treated substrates to enzymatic hydrolysis was demonstrated by enzymatic digestibility and the results are shown in Fig. 3. The integrated process based on SEP and alkaline post-treatment could effectively improve the enzymatic digestibility of bamboo stems in this study. The cellulose digestibility was just 5.8% for the untreated substrate after 72 h enzymatic hydrolysis. After SEP, this value reached to 17.1–32.2%. As the pretreatment severity increased, the cellulose digestibility of the steam-exploded substrates (S3, S4, and S5) increased, which was probably due to the gradual disintegration of the substrates, especially at the harsh condition (2.0 MPa, 8 min). As expected, the cellulose digestibility of S2 reached a maximum value (32.2%) in all the steam-exploded substrates, suggesting that direct SEP was more effective than SEP with KOH presoaking for producing glucose by enzymatic hydrolysis. These results were in agreement with the effects of SEP on bamboo stems, such as the removal of hemicelluloses, partial removal of lignin, partial relocation of lignin, increased surface area, and loose degree of surface morphology; these are all deemed favorable in increasing the enzymatic digestibility of cellulose to glucose.

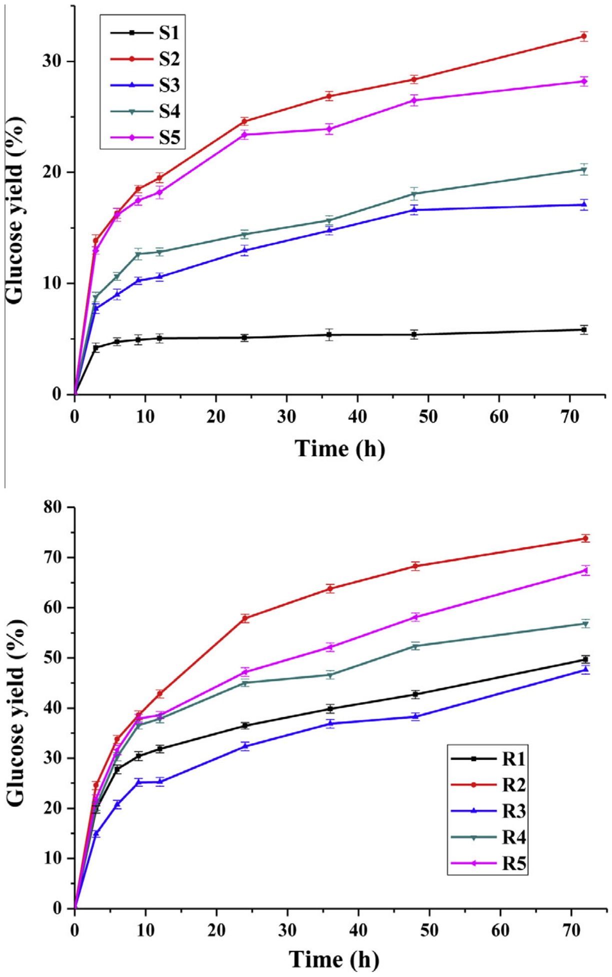

Fig. 3. Glucose yields of enzymatic hydrolysis of the untreated, steam-exploded, and further alkali/alkaline ethanol post-treated substrates.

For the alkaline post-treated substrates, the cellulose digestibility of the untreated substrate was 49.7%, whereas the cellulose digestibility of the steam-exploded substrates increased to 56.8–73.8%. For substrates R3 and R4, as the pressure of SEP increased, the cellulose digestibility of the substrates increased. Likewise, the cellulose digestibility of the substrates increased with the increased time of SEP (R4 and R5). Interestingly, a maximum cellulose digestibility (73.8%) was detected in R2. The significant increment of cellulose digestibility of the substrates after alkaline post-treatment was mainly attributed to the effective removal of hemicelluloses and lignin from the pretreated substrates by alkaline post-treatment. Recently, the influence of pressure and time of SEP on the delignification and structural features of lignin with alkaline treatment had been investigated [21]. The results showed that the increasing pressure and time of SEP removed more lignin from the steam-exploded bamboo stems. Hence, the increasing removal amount of lignin was positively correlated with an increasing enzymatic digestibility of cellulose. In short, delignification by the alkaline treatment could improve the enzymatic digestibility of the substrate.

3.7. The relationship between physiochemical properties and enzymatic hydrolysis

The physiochemical (e.g., chemical compositions and morphology) variations of lignocelluloses are the important factors that can influence their enzymatic digestibility [34]. To clarify the effect of the physiochemical changes of the substrates after different treatments on the enzymatic digestibility of cellulose, the relationship between the enzymatic digestibility and physiochemical changes should be discussed. As can be seen from Fig. 4, the optimal enzymatic digestibility was observed in S2 and R2, respectively. The effective conversion was mostly affected by the content of hemicelluloses, similar result was obtained in a recent publication [35]. These factors were also suggested that the decrease of hemicelluloses and lignin favors the enzymatic digestibility of the pretreated substrates. The strong positive correlations between delignification degree and the rate of enzymatic hydrolysis have been observed in a previous study [36]. Thus, it was concluded that the removal of hemicelluloses and lignin during the SEP and post-treatment processes would facilitate cellulase access to the cellulose, resulting in increased enzymatic digestibility of cellulose.

The relationship between CrI and enzymatic digestibility has been extensively studied [37,38]. Although the CrIs of the substrates were detected by the researchers in most of cellulose digestibility experiments, the relationship between CrI and enzymatic digestibility has not been comprehensively investigated. In general, the CrI was positively related to the enzymatic digestibility if no transformations of XRD patterns occur, such as the formation of amorphous cellulose or cellulose II from cellulose I. In these

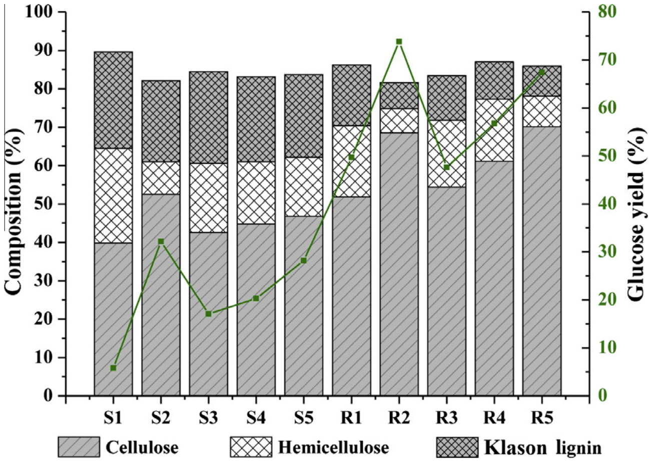

Fig. 4. Relationship between chemical compositional distribution and enzymatic digestibility of substrates.

cases, the CrI was positively related to the enzymatic digestibility of cellulose, as revealed by the data presented in this study. However, if the CrI was decreased after specific pretreatment, which induced the changes of XRD patterns of cellulose, favoring the formation of amorphous cellulose or cellulose II from cellulose I. Under these conditions, the pretreated substrates were more readily digested than cellulose I, such as under ionic liquid pretreatment and phosphoric acid pretreatment [29,39]. Based on these observations, it was found that the crystallinity was not an important factor as generally believed. In fact, the increase in hydrolysis efficiency after alkaline treatment was probably due to the removal of amorphous material rather than the increase in the ordered region.

Based on the data presented in Table 2, Figs. 2 and 3, the SEM images demonstrated that the SEP could cause severe degradation and evident damage for the intact cell structure, thus resulting in improved enzymatic digestibility rates of the steam-exploded substrates. It was also demonstrated that the combination of SEP and alkaline treatment was a promising approach for the effective removal of hemicelluloses and lignin, and thus increased the enzymatic digestibility of cellulose [21]. The enzymatic hydrolysis rates of the substrates were enhanced with the increment of the BET surface areas, which was in agreement with the published literature [31]. Moreover, it was reported that the enzymatic digestibility was in strong and positive correlation to the available surface area [40,41]. That is, the smaller particles of substrates were hydrolyzed more efficiently by cellulase. Hence, size-reduction by milling or refining was very necessary for the improvement of enzymatic digestibility of cellulose in the biorefinery industry.

4. Conclusions

A combined system (steam explosion pretreatment followed by mild alkaline post-treatment) was applied to enhance enzymatic digestibility of bamboo stems. After SEP, the morphologic structure of bamboo stems was broken up and the cellulose bundle surface was exposed with the increase of the pretreatment pressure and time, which was favorable for cellulase enzyme absorption on cellulose. After further alkaline treatment, cellulose was significantly hydrolyzed by cellulase to glucose for bioethanol production while lignin was recovered after alkaline treatment for further utilization. The maximum enzymatic digestibility of cellulose of 73.8% was achieved after the pretreatment under direct SEP condition and subsequent alkaline treatment. Thus, the SEP coupled with alkaline post-treatment could be considered as a promising approach to achieve the efficient fractionation and conversion the major components of bamboo stems into various bio-based products in the biorefinery process.

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Appendix A. Supplementary material

Supplementary data associated with this article can be found, in the online version, at http://dx.doi.org/10.1016/j.apenergy.2014.09.068.

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